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Journal of Chromatography A, 684 (1994) 323–328

JOURNAL OF CHROMATOGRAPHY A

Influence of the capillary edge on the separation efficiency in capillary electrophoresis

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First received 21 March 1994; revised manuscript received 14 June 1994

Abstract

The results of the present work show that the physical shape of the injection end of the capillary in CE has a major effect on the observed efficiencies. Straight-edge capillaries yield much more efficient electropherograms than do slanted edge capillaries. Also, injections into straight-edge capillaries give more symmetrical peaks. Indications are that the effect of the inlet shape is independent of molecular size. Moments analysis shows that the increase in size of the injected plug, due to the slanted shape of the capillary inlet, is not sufficient to explain the large decrease in efficiency.

1. Introduction

Separation efficiencies in capillary electrophoresis (CE) which are attainable in practice are often smaller than the theoretically predicted values. This loss in efficiency can be the result of extra broadening processes such as wall adsorption of solutes, parabolic flow profile due to temperature gradients etc. (e.g., [1,2]). Among these processes, excessive zone broadening due to sample introduction can be the dominating factor in the total zone dispersion.

While there are several methods of introducing solutes into the capillary in CE, the two most common injection techniques use either electrokinetic migration or pressure displacement. In both methods the injection protocol involves several manipulations of the running buffer and sample vials. Thus, the injection process is cumbersome and, intuitively, prone to problems.

There are several reports on difficulties related to sample introduction. Huang et al. [2], Burton et al. [3] and Terabe et al. [1] found that the injection length affects the efficiency. Rose and Jorgenson [4] compared the above two injection methods. While they could not find any significant difference in the efficiency between the two, they did notice a difference in peak size that they attributed to preferential sample introduction in the electrokinetic approach. Grushka and McCormick (5) described a ubiquitous sample introduction that resulted from the actual immersion of the capillary in the sample vial. This ubiquitous injection occurs over and above the conventional injection. The size of sample plug thus entering the capillary is quite large, resulting in lower than expected efficiencies. Schwartz et al. [6] confirmed the presence of the ubiquitous injection. Recently, Fishman et al. [7]

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0021-9673/94/$07.00
SSDI 0021-9673(94)00597-3

showed that the ubiquitous injection is caused by an interfacial pressure difference formed at the inlet of the capillary. They also discussed means of eliminating or greatly diminishing this undesirable mode of injection. Lux et al. [8] found that the injection process produces asymmetric peaks, which can be eliminated by rinsing the outer part of the capillary with clean buffer after the sample introduction part of the injection process. Dose and Guiochon [9] discussed the effects of diffusion and hydrodynamic flow on the amounts of solutes entering the capillary during the injection process.

In the present communication we show that the quality of the injection depends strongly on the physical condition of the inlet side of the capillary.

2. Experimental

2.1. Apparatus

The instrument used was a laboratory-made CE unit. A high-voltage power supply (Glassman, NJ, USA) was used to establish the electrical field across the capillary. The output voltage of the power supply was computer controlled. Capillaries were polyimide coated fused silica (Polymicro Technologies, CA, USA) 50 μm I.D. and 375 μm O.D., having separation lengths varying from 38 to 42 cm and total lengths from 72 to 74 cm. Detection was done with a Model 200 UVIS absorbance detector (Linear Instruments, CA, USA) at 280 or 200 nm for proteins. A section of about 1 cm of the capillary coating was removed by heat, to serve as a UV-detection window. The signals from the detector were fed to a Model 600 recorder (W + W Electronic, Switzerland) and a Model D-2000 Hitachi integrator (Merck, Germany).

2.2. Reagents

Buffers

The following buffers were used: (a) 0.02 *M* NaH_2PO_4 adjusted to pH 6 with NaOH (Frutarom, Israel), (b) 0.02 *M* sodium acetate (Merck) adjusted to pH 5 with acetic acid (Frutarom) and (c) 0.02 *M* Na_2HPO_4 (Mallinckrodt, USA) adjusted to pH 11 with NaOH.

Additives

The running buffer contained either 0.01 *M* triethylamine (TEA) or 0.001 *M* isoleucine as additives. The additives are used to maintain constant electroosmotic flow to ensure constant migration times [10].

Solutes

The solutes used were phenol (Mallinckrodt) and myoglobin (Sigma, MO, USA). The solutes were dissolved in the running buffer.

2.3. CE procedures

Injection of the sample into the capillary was made by electromigration at 5 kV for 1 s. Electrophoresis was run at 25 kV applied voltage. The current through the capillary did not exceed 50 μA.

2.4. Capillary treatment

Each new capillary was cleaned by flushing, sequentially, with 1 *M* KOH (Frutarom) for 15 min, triply distilled water for 30 min and the running buffer which contained an additive for a few seconds. The capillary was conditioned for 3 h, at 25 kV, to allow equilibration and consistency of migration times. This treatment eliminates the need for capillary washing between runs.

2.5. Preparation of the injection end of the capillary

The capillary edge was cut, usually with scissors, either on a slant or straight. Often, the capillary was cut having one shape, and after a few electrophoretic runs it was recut to give the other shape. The capillary edges were photographed through an Olympus Model SZH microscope at $\times 64$ magnification.

3. Results and discussion

In our studies in CE we found that when we changed capillaries we could not reproduce peak shapes and, consequently efficiencies, for identical systems; i.e., same running buffers, solutes, injection conditions and applied voltages. Washing the injection end of the capillary after the injection process, as suggested by Lux et al. [8], did not seem to alleviate the problem since the phenomenon was dependent mainly on the capillary and not the actual injection process. While trying to establish whether the polyimide coating had an effect on the efficiency, we examined the capillary edge with a microscope. It became apparent that the capillary glass shape is the important factor.

A capillary with a straight injection end is shown in Fig. 1a and with a slanted end in Fig. 1b. An electropherogram of phenol obtained with the straight-cut capillary is given in Fig. 2a and one from the capillary with the slanted injection edge in Fig. 2b. The capillary in Fig. 1b is the same as in Fig. 1a but it was cut to give a slanted edge. The solute velocity was identical in both capillaries. The peak obtained with the straight-edge capillary is narrower than that obtained with the slanted-edge capillary; the plate numbers are 161 000 and 122 000, respectively.

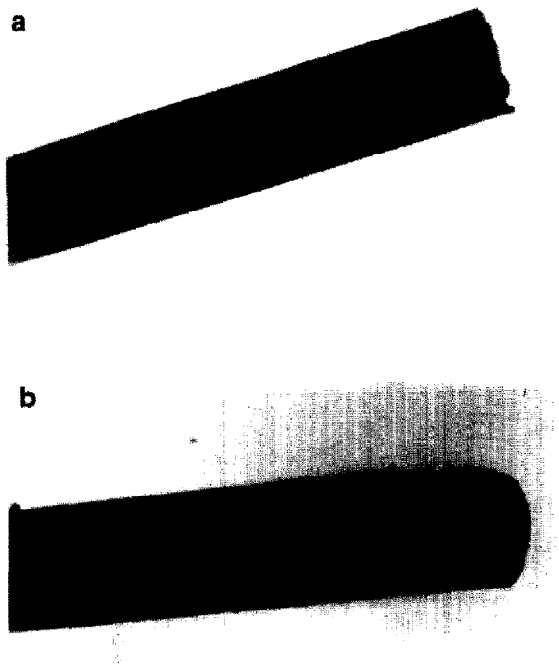

Fig. 1. Photographs of the injected end of a 50 μm I.D. 375 μm O.D. capillary used with phenol as solute. (a) Straight-edge capillary; (b) slanted-edge capillary.

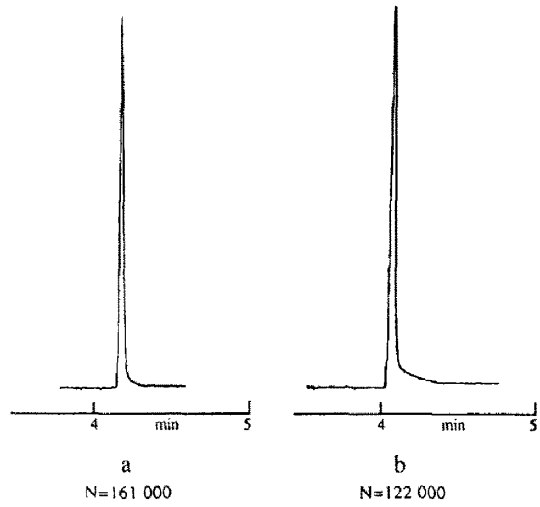

Fig. 2. Electropherograms of phenol, (a) obtained with the capillary in Fig. 1a and (b) obtained with the capillary in Fig. 1b. The electrophoresis conditions were: applied voltage 25 kV; 0.02 *M* NaH_2PO_4 buffer at pH 6; 0.01 *M* triethylamine added to the buffer to maintain constant electroosmotic flow.

Similar results were obtained with all buffers and additives mentioned in the Experimental section. For example, Fig. 3 gives a series of electropherograms of phenol, obtained under various conditions (different pH values and different additives) using several capillaries. The peaks on the right side of the figure resulted from injection into slanted-edge capillaries whereas the peaks on the left side are from the straight-edge capillaries. Injection into a straight-edge capillary resulted not only in increased efficiency but also in better peak symmetries.

The above results were obtained with small solutes. Similar results are observed with large solutes as well. As an example, Fig. 4a gives the electropherogram of the protein myoglobin (phosphate buffer at pH 11) obtained with the straight-edge capillary shown on the left side of Fig. 5. The result with the slanted-edge capillary (right side of Fig. 5), is shown in Fig. 4b. The

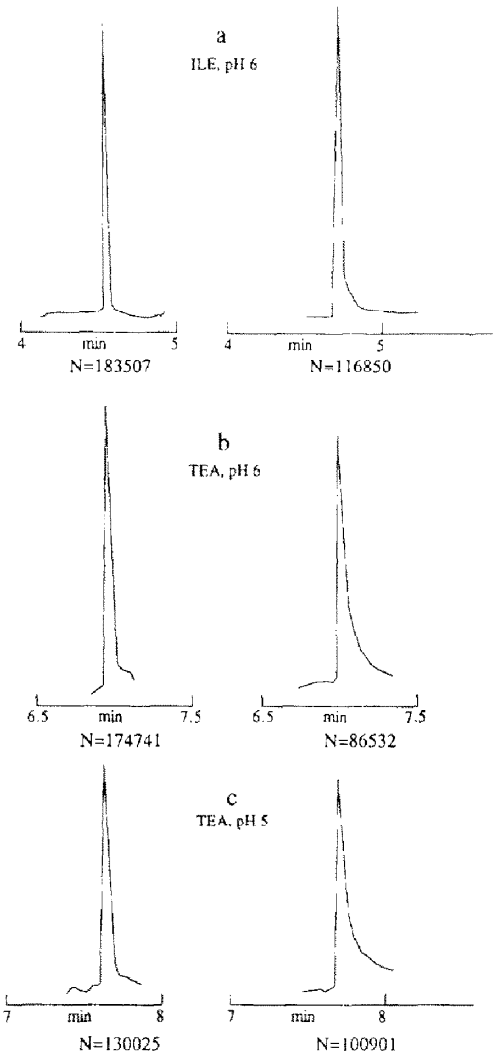

Fig. 3. Electropherograms of phenol under different conditions: (a) 0.02 *M* NaH_2PO_4 buffer pH 6 plus 0.001 *M* isoleucine additive, (b) 0.02 *M* NaH_2PO_4 buffer pH 6 plus 0.01 *M* TEA additive and (c) 0.02 *M* CH_3COONa buffer pH 5 plus 0.01 *M* triethylamine additive. In each case the right electropherogram was obtained with a slanted-edge capillary.

plate number for the peak in Fig. 4b is 87 000 as compared to 230 000 for the peak in Fig. 4a. The plate number for Fig. 4a is much lower than that predicted, most likely due to interaction of the protein with the capillary wall, even at pH 11. The peak in Fig. 4b is much smaller than that in Fig. 4a although both samples were injected under identical conditions. The small impurity

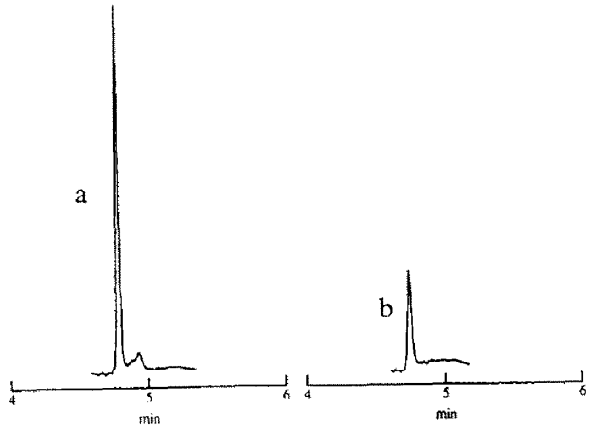

Fig. 4. Electropherograms of myoglobin; 0.02 *M* Na_2HPO_4 buffer, pH 11, 0.01 *M* TEA, 25 kV. (a) Straight-edge capillary; (b) slanted-end capillary.

peak evident in Fig. 4a is not observed in Fig. 4b due to the additional broadening effect of the slanted edge.

The peak-shape dependency on capillary edge geometry was examined by us with several different running buffers, with several different additives (used for electroosmotic flow control) and with several solutes (both small and large). Results similar to those observed above were obtained with all systems which we studied.

Our results indicate that the physical shape of the capillary end does influence the efficiency of the separation. Undoubtedly, the capillary edge affects the size of the injected sample plug. Fig. 6a shows, in a schematic manner, the shape of a sample plug obtained from a straight-edge capillary. The contribution of that sample zone to the overall peak variance is [11]:

$$\sigma_{inj}^2 = \frac{l^2}{12} \quad (1)$$

Fig. 5. The capillary with which the electrophoresis of myoglobin was done. Shown are the straight edge on the left and the slanted edge on the right.

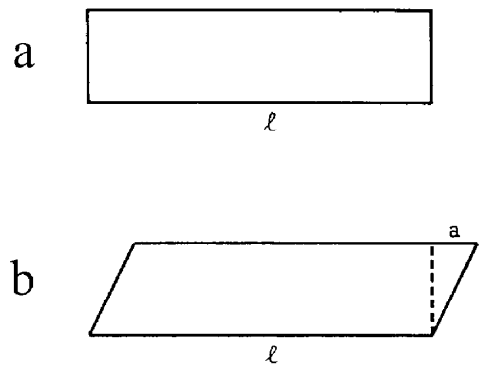

Fig. 6. Schematic representation of the injected plug. (a) Plug resulting from a straight-edge capillary; (b) plug resulting from a slanted-edge capillary.

σ^2_{inj} is the plug variance and l is the plug length. Fig. 6b represents the injected zone shape resulting from a slanted edge. The contribution here to the peak variance is:

$$\sigma^2_{inj(slant)} = \frac{1}{12} \cdot (l^2 + a^2) \quad (2)$$

where a is the extra length in the injection zone (Fig. 6b). Therefore, the increase in the size of the injected plug due to the slanted edge of the capillary is $a^2/12$.

The length of the parameter a depends on the angle of the slant. Simple trigonometric calculation shows that, for a 50 μm I.D. capillary, parameter a is 87, 50 and 29 μm for a slant angle of 30, 45 and 60°, respectively. In a straight-edge capillary, a typical size of the injected zone is about 350 μm. Thus, according to Eq. 2, the variance of the injected plug will increase only by about 10% for the most unfavorable slant angle of 30°. That modest increase in the variance of the injected sample does not explain the large decrease in efficiency that is observed in practice. If we assume that, due to diffusion, the injected zone will revert quickly to a plug shape having the length of $l + a$, the increase in the injected zone variance is still not sufficient to explain the large decrease in the observed efficiency.

Even with the straight-edge capillary, the plate height values are smaller than theoretically expected, based on estimated diffusion coefficient values of the solutes. For example, based on its size, the diffusion coefficient of myoglobin is estimated to be about $1 \cdot 10^{-6}$ cm^2/s [12]. Hence, the plate height for conditions similar to those in Fig. 4 should be around 0.12 μm. In practice we obtained 1.9 μm with a straight-edge capillary. With phenol as solute, the theoretical H value, for the conditions of Fig. 3, are around 1.2 μm as opposed to an observed value of 2.4 μm with a straight-edge capillary. Thus, in addition to molecular diffusion, there are other processes that add to the width of the solute zone. Still, even though the observed efficiency is lower than the prediction, the effect of the shape of the injection end of the capillary is most pronounced.

As discussed before, the increase in the size of the injected zone is not sufficient to explain the profound effect of the capillary edge shape. Other causes must be responsible for the large decrease in efficiency. The exact nature of these causes is not clear to us at the present stage. We do not feel that the decrease in efficiency can be explained by interfacial pressure difference, which seems to control sample penetration in CE [7]. Undoubtedly, the slanted edge causes a current density difference between the capillary inlet and outlet. This gradient in current density can effect electroosmotic and electrophoretic mobilities. A gradient in electroosmosis can in turn cause excessive zone broadening. In addition, because part of the capillary in the slanted inlet is not bound by a wall (as is the case in the actual capillary after the injection edge) the electroosmosis flow profile may be greatly distorted, as bulk liquid from the reservoir is trying to enter the capillary. This distortion in the electroosmosis will also have deleterious effects on the efficiency and on peak shape.

The exact mechanism by which the zone broadens due to slanted capillary inlet is yet to be found. However, the effect is real as judged from its observed reproducibility: relative standard deviation of $< 1\%$ in migration times and $< 2\%$ in H values. It is clear that more careful attention must be given to the exact shape of the capillary inlet. A straight-edge capillary inlet is

essential for high efficiencies in CE separations as well as for more symmetrical peaks.

Acknowledgements

We wish to thank Dr. Gil Shoham and Ms. Hadar Feinberg for their help photographing the capillaries. This research was supported by grant No. 88-00021 from the United States–Israel Binational Science Foundation (BSF), Jerusalem, Israel.

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